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## Original Paper

# Chemosensitivity of Prostate Cancer Cell Lines and Expression of Multidrug Resistance-related Proteins

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**The aim of this study was to obtain insight into the role of the multidrug resistance (MDR) phenomenon in hormone-independent progressive prostate cancer. Using immunocytochemistry and Western blotting we determined the expression of P-glycoprotein (Pgp), multidrug resistance-associated protein (MRP), glutathione-S-transferase-π (GST-π), Bcl-2, Bax, topoisomerase (Topo) I, IIα and IIβ in the human prostate cancer cell lines PC3, TSU-Pr1, DU145 and LNCaP derivatives LNCaP-R, LNCaP-LNO and LNCaP-FGC. Proliferative activity was assessed by immunocytochemistry. MTT assays were used to determine the sensitivity to etoposide, doxorubicin and vinblastin. Pgp was not expressed in any of the cell lines. MRP was variably expressed. GST-π was expressed in TSU-Pr1, PC3 and DU145. The expression of Bcl-2 was restricted to TSU-Pr1, whereas Bax was found in all cell lines. Topo IIα was expressed at the highest level in the rapidly proliferating cell lines TSU-Pr1 and DU145. Topo I and IIβ were equally expressed. Resistance profiles varied among the cell lines, with TSU-Pr1 being the most sensitive and LNCaP-LNO relatively resistant. Multiple MDR proteins were expressed in prostate cancer cell lines and may well influence response to chemotherapy. Future functional studies, using chemo-selected MDR models, may further help to determine the mechanism or combination of mechanisms underlying the resistance of prostate cancer to chemotherapy.** © **1999 Elsevier Science Ltd. All rights reserved.**

**Key words: multidrug resistance, prostate cancer, chemosensitivity, cell lines, hormone-independent prostate cancer, chemotherapy**
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## INTRODUCTION

PROSTATE CANCER is the most common cancer in men and one of the leading causes of cancer death [1]. Metastatic disease is a major oncological problem and is not curable, but palliation can be achieved by hormone deprivation therapy [2]. However, prostate cancer patients will relapse at some stage in time, the majority within 1–2 years after starting endocrine therapy. After failure of hormone therapy, there are no successful treatment strategies available with respect to prolongation of survival and patients will die of progressive, hormone-independent disease after an average period of 40 weeks following relapse [3].

Hormone-independent prostate cancer is resistant to a broad range of antineoplastic agents [4], which may be caused by the fact that large proportions of prostate cancer cells are in interphase [5]. Also, multidrug resistance (MDR), the resistance of cancer cells to a variety of structurally and functionally distinct cytotoxic agents, may play an important role in progressive therapy resistant prostate cancer. However, the role of MDR in prostate cancer remains to be elucidated. A better understanding of mechanisms underlying the resistance of prostate cancer to chemotherapy may lead to novel approaches to challenge hormone unresponsive prostate cancer more successfully.

We determined the expression of eight MDR-associated proteins in prostate cancer cell lines derived from patients with progressive disease: the drug transporter molecules P-glycoprotein (Pgp) [6] and MDR-associated protein (MRP [7], the detoxifying enzyme glutathione-S-transferase-π (GST-π) [8], modulators of apoptosis Bcl-2 [9] and Bax [10] and the enzymes topoisomerase (Topo) I, IIα and IIβ, which

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are related to one form of MDR: atypical or attenuated MDR [11]. Furthermore, we measured the response of the cell lines to treatment with the cytotoxic agents etoposide, doxorubicin and vinblastin, which are commonly used in the treatment of several malignancies and have been used to challenge prostate cancer in clinical trials.

## MATERIALS AND METHODS

*Cell lines and culture conditions*

The human prostate cancer cell lines PC3 [12], TSU-Pr1 [13], DU145 [14] and LNCaP sublines that were established from the original LNCaP line [15]: the androgen-dependent FGC (available through the ATCC, Rockville, Maryland, U.S.A.), the hormone unresponsive R line [16] and the hormone-independent LNO line [17], were maintained at 37°C in a 5% $CO_2$–95% air atmosphere. PC3, TSU-Pr1, DU145, LNCaP-R and LNCaP-FGC were cultured in RPMI 1640 medium, supplemented with 7.5% fetal calf serum (FCS), 1 unit/ml penicillin, 1 μg/ml streptomycin and 2 mM glutamine (GibcoBRL). The culture medium for LNCaP-LNO contained 5% dextran-coated charcoal (DCC) treated (androgen depleted) serum, instead of FCS. All cell lines were repeatedly tested for *Mycoplasma* by polymerase chain reaction and proven to be free of infection.

*Antibodies*

For the detection of Pgp the mouse monoclonal antibody JSB1 (Sanbio, The Netherlands) was used. MRP was detected with the rat monoclonal antibody MRPr1 (Sanbio), GST-π with the rabbit polyclonal antibody NCL-GST-π (Novocastra, U.K.), Bcl-2 with the mouse monoclonal antibody Bcl-2 (100) (Santa Cruz Biotechnology Inc., California, U.S.A.), Bax with the rabbit polyclonal antibody Bax (P-19) (Santa Cruz Biotechnology), Topo I with the human Scl-70 antibody (Topogen Inc., Columbus, Ohio, U.S.A.). Topo IIα with the mouse monoclonal antibody Ki-S1 (Boehringer Mannheim, Germany). Topo IIβ was detected with a polyclonal rabbit antibody (Biotrend, Köln, Germany) for Western blotting and the monoclonal mouse antibody 3H10 (a kind gift from I. Hickson, Cambridge, U.K.) for immunocytochemistry. BrdU was detected with the mouse monoclonal antibody 2B5 (Eurodiagnostics, The Netherlands). Horseradish peroxidase (HRP)-conjugated secondary antibodies were all purchased from Dako (Glostrup, Denmark).

*Antineoplastic drugs*

For *in vitro* testing, drug solutions were freshly prepared in culture medium. The following compounds were tested: etoposide (Pharmachemie BV, Haarlem, The Netherlands), doxorubicin (Farmitalia Carlo Erba, Italy) and vinblastin (Lilly, France).

*MTT assay*

The sensitivities of all cell lines to the antineoplastic drugs were assessed using the MTT assay [18]. Cell lines were plated in 96-well plates (Costar Corp., Cambridge, Massachusetts, U.S.A.) at densities allowing logarithmic growth throughout the experiments. After allowing cells to attach for 24 h, proliferating cells were incubated with culture medium containing a range of exponentially increasing concentrations of cytotoxic agents for a period of 72 h. Subsequently, 30 μl of MTT (Sigma Chemical Co., St Louis, Missouri, U.S.A.) solution (5 mg/ml in phosphate buffered saline (PBS)) was added to each well and incubated for 4 h at 37°C. The plates were centrifuged at 1500 rpm for 5 min to precipitate non-adherent and loosely adherent cells. The supernatant was carefully aspirated and 100 μl of dimethylsulphoxide (DMSO, Merck, Darmstadt, Germany) with alkaline buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) was added to each well. The plates were shaken for 5 min in order to dissolve the formazan crystals. The absorbance was measured photometrically at 570 nm using a BioRad Microplate Reader (Model 450, BioRad, California, U.S.A.). The percentage of viable cells was calculated relative to untreated cells. All assays were performed in triplicate. $IC_{50}$ values for the separate drugs were calculated from the dose–response curves by interpolation. Standard errors of the mean (SEM) were calculated from the square root of the variance as determined with the Delta method [19].

*Western blotting*

Whole cell lysates were prepared from near-confluent cell cultures ($1\times10^6$ cells). Separate protocols, optimised for the detection of membrane and cytoplasmic proteins Pgp and MRP, cytosolic protein GST-π, cytoplasmic proteins Bcl-2 and Bax and the nuclear proteins Topo I, IIα and IIβ, were used for the preparation of lysates. For Topo detection, cells were scraped and resuspended in lysis buffer (10 mM Tris–HCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, pH 7.8) containing protease inhibitors (1 mM phenylmethylsulphonylfluoride, 1 mM benzamidine, 10 mg/ml soy bean trypsin inhibitor, 50 mg/ml leupeptin, 1 μg/ml pepstatin, 20 μg/ml aprotinin; Sigma) and put on ice for 20 min. Then, 120 units of DNase I (Sigma) were added and incubated for 20 min at 37°C. For Bcl-2 and Bax detection, cells were scraped and resuspended in another lysis buffer (10 mM Tris–HCl, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 5 mM ethylane diamine tetracetic acid (EDTA), pH 7.4) supplemented with the aforementioned protease inhibitors. The suspension was kept on ice for 15 min, subsequently centrifuged (14 000 rpm) for 20 min at 4°C and the supernatant recovered. For Pgp and MRP detection, harvested cells were resuspended in lysis buffer (10 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl, 0.5 (w/v) SDS, pH 7.4) supplemented with protease inhibitors. DNA was sheared by sonification. For GST-π, near-confluent cultures were trypsinised and homogenised in 2.5 mM Tris-HCl buffer (pH 7.5) containing 0.25 mM EDTA, 1.25 mM EGTA and 31.3 mM sucrose. After two serial centrifugation steps of 10 min at 1000 ***g*** and 1 h at 105 000 ***g*** the supernatant was recovered. Protein concentrations were measured (BioRad protein assay). All cell lysates were stored at − 80°C. Whole cell lysate samples (40 μg protein) with Laemmli buffer [20] (15 μl) were fractioned on to a 10% SDS–polyacrylamide gel after boiling for 2 min (except the Pgp and MRP lysates) and electrophoresed for 45 min at 200 V. Prestained markers (Novex, San Diego, California, U.S.A.) were used as size standards. Proteins were transferred to a nitrocellulose membrane (Protan Nitrocellulose, Schleicher & Schuell, Dassel, Germany) by electroblotting at 100 V. The membranes were blocked for 1 h with Tris buffered saline (TBS, pH 7.5) containing 1% blotting substrate (Boehringer Mannheim). Separate membranes were incubated overnight at 4°C with antibodies directed to the specific MDR proteins. After washing with TBS containing 0.1% Tween 20 (Sigma), the membranes were incubated with the appropriate

peroxidase-conjugated secondary antibodies (see Antibodies in Materials and Methods). The protein bands were visualised by chemiluminescence. After incubation with detection solution (Boehringer Mannheim) X-ray film (Fuji photo film, Tokyo, Japan) was exposed and developed.

*Immunocytochemistry*

Cells were grown on 3-aminopropyl-trietoxysilane (Sigma) coated glass slides until semiconfluent cultures were obtained. For BrdU staining, cells were incubated with bromodeoxyuridine (BrdU, Sigma) for 2 h. To obtain optimal availability of the antigen for detection with the antibody, the cells were fixed according to the manufacturers' instructions for the use of the various antibodies. After fixation in acetone (Pgp), 3.7% paraformaldehyde (MRP, GST-π, Bcl-2, Bax, Topo I, IIα and IIβ) or 70% ethanol (BrdU), endogenous peroxidase activity was blocked with 1% hydrogen peroxide (Merck) in 100% methanol. For BrdU detection, slides were incubated with 2N HCl followed by 0.1 M Borax buffer. The slides were then placed in a humid incubator (Sequenza, Shandon, U.K.) and incubated with normal goat or rabbit serum (Dako) diluted 1:10 in PBS/5% bovine serum albumin (Sigma). Subsequently, the slides were incubated overnight with antibodies directed against either Pgp, MRP, GST-π, Bcl-2, Bax, Topo I, IIα, IIβ or BrdU. For Pgp, Bcl-2, Topo IIα and Topo IIβ the peroxidase–antiperoxidase (mouse-PAP, Dako) method was applied to increase staining sensitivity. The slides were incubated with appropriate HRP-conjugated secondary antibodies (all from Dako) and the antigen–antibody binding was visualised with 0.075% 3,3′-diaminobenzidine-tetrahydrochloride (Fluka, Germany) and 0.25% hydrogen peroxide in PBS. The slides were counterstained with haematoxylin, dehydrated and covered. Negative controls were included for all stainings by replacing the primary antibody by PBS.

## RESULTS

*Chemosensitivity assay*

Drug-induced inhibition of human prostate cancer cell growth by the anticancer agents etoposide, doxorubicin and vinblastin was established *in vitro*. The growth of the cell lines during the assay, defined as the optical density (OD) value

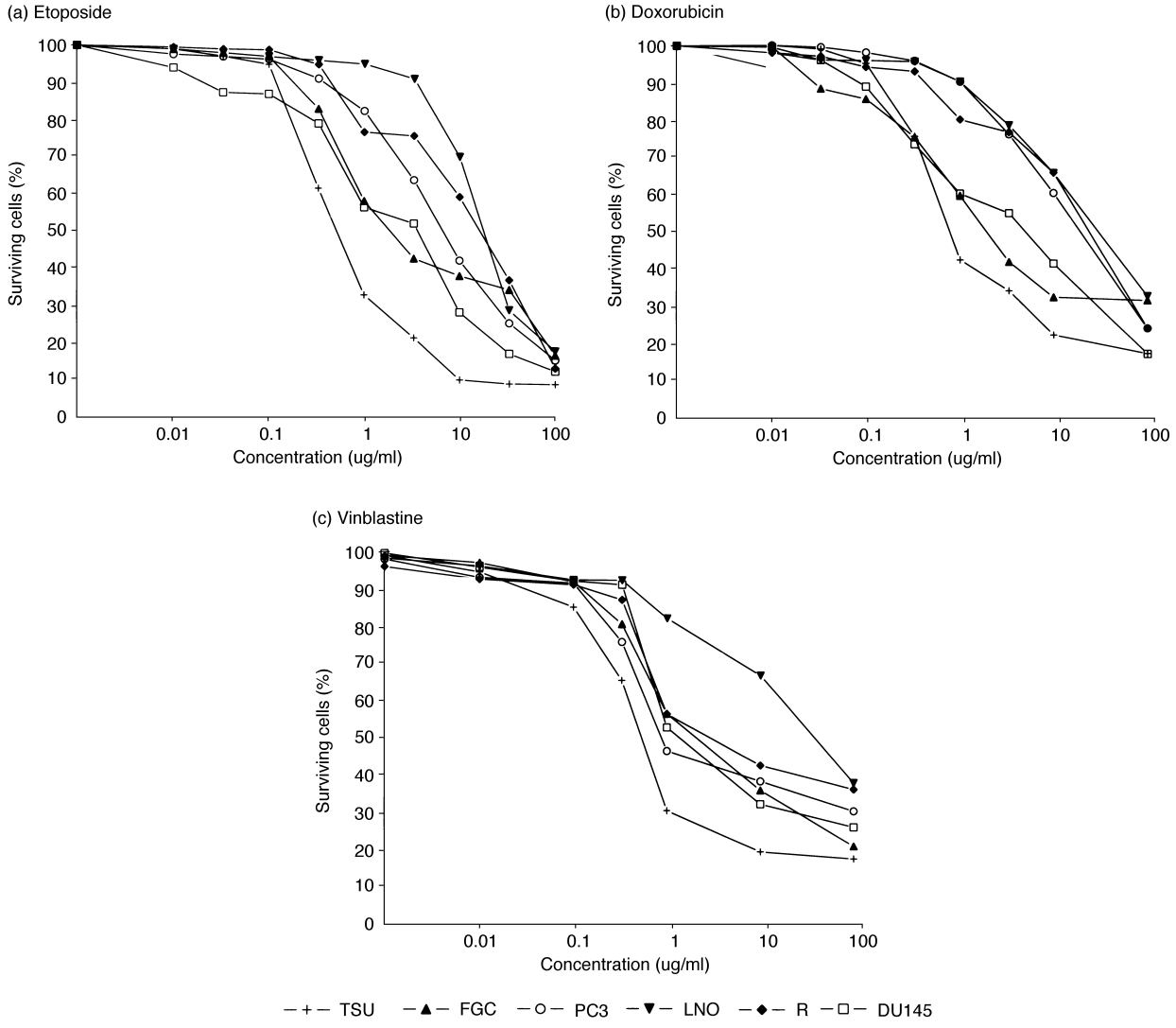


**Figure 1. Dose–response curves of human prostate cancer cell lines TSU-Pr1, LNCaP-FGC, PC3, LNCaP-LNO, LNCaP-R and DU145 after 72 h of exposure to (a) etoposide, (b) doxorubicin and (c) vinblastine. Values are given as a percentage of the untreated controls. *y*-axis, percentage of viable cells as calculated relative to untreated controls ($n = 24$); *x*-axis, concentrations of the drug solutions on a log scale.**

*Table* 1. *$IC_{50}$ values of human prostate cancer cell lines as determined with the MTT assay**

| | TSU-Pr1 | LNCaP-FGC | PC3 | LNCaP-LNO | LNCaP-R | DU145 |
|---|---|---|---|---|---|---|
| Etoposide (μg/ml) | 0.48 ± 0.05 | 1.9 ± 0.14 | 6.1 ± 0.15 | 21 ± 0.4 | 16 ± 0.6 | 3.6 ± 0.1 |
| Doxorubicin (ng/ml) | 7.2 ± 0.2 | 16.7 ± 0.2 | 176 ± 7.4 | 272 ± 4.5 | 222 ± 10 | 41 ± 2.5 |
| Vinblastin (ng/ml) | 0.5 ± 0.01 | 1.8 ± 0.2 | 0.8 ± 0.02 | 35 ± 2 | 2.4 ± 0.2 | 1.2 ± 0.1 |

*$IC_{50}$ values are calculated by interpolation, standard errors of the mean are calculated by taking the square root of the variance of the $IC_{50}$ value as determined by the Delta method [19].

after 72 h divided by the OD value after plating of the cells, was 5.4, 2.6, 3.3, 1.5, 3.3 and 3 for TSU-Pr1, LNCaP-FGC, PC3, LNCaP-LNO, LNCaP-R and DU145, respectively. This indicates that drug testing was performed under the condition of cell lines in proliferative growth. Dose–response curves of each cell line for the three drugs tested are shown in Figure 1 and $IC_{50}$ values are given in Table 1. The cell lines showed a variable response to a 72 h exposure to etoposide: TSU-Pr1 was the most sensitive, followed by DU145 and LNCaP-FGC. PC3 had an intermediate pattern of response, whereas LNCaP-LNO and LNCaP-R were relatively insensitive (Figure 1a). The sensitivity profiles of the cell lines with doxorubicin were similar to those found for etoposide, as shown in Figure 1b. The MTT assay with vinblastin (Figure 1c) showed that TSU-Pr1 was the most sensitive followed by PC3 and DU145. The LNCaP derivatives were less sensitive: LNCaP-FGC was nearly as sensitive as DU145, but LNCaP-R was 2-fold more resistant and LNCaP-LNO was relatively insensitive to vinblastin.

*Western blotting*

Pgp, MRP, GST-π, Bcl-2, Bax, Topo I, IIα and IIβ were detected by immunoblotting of total cell lysates. All experiments were performed in triplicate and reproducible results were obtained. Representative protein bands are shown in Figure 2. The expression of Pgp was below detection levels in all cell lines. The Pgp positive, MDR cell line RC21E [21], served as a positive control and was run on the same gel. MRP was variably expressed, but at much lower levels than in the control sample, the human MDR lung cancer cell line GLC4/ADR [22] (not shown). GST-π was clearly expressed in the cell lines TSU-Pr1, PC3 and DU145, whereas LNCaP-FGC, LNCaP-LNO and LNCaP-R had no detectable levels of GST-π, with the colon carcinoma cell line HT-29 [23] serving as a positive control. The expression of Bcl-2 was restricted to TSU-Pr1, whereas Bax was found in all cell lines. Bcl-2 expression in TSU-Pr1 was low in comparison with Chinese hamster ovary cells transfected with Bcl-2 (kindly provided by H. Burger, Department of Oncology, Erasmus University, The Netherlands), which was run on the same gel. The Topo proteins were expressed in all cell lines. The Topo IIα isoform was most clearly expressed in the rapidly proliferating cell lines TSU-Pr1 and DU145. The IIβ isoform was uniformly expressed in all cell lines, which also applied to the Topo I protein.

*Immunocytochemistry*

In addition to Western blot analysis, cultured prostate cancer cells were analysed for the expression of the same MDR proteins by immunocytochemical staining. Near-confluent

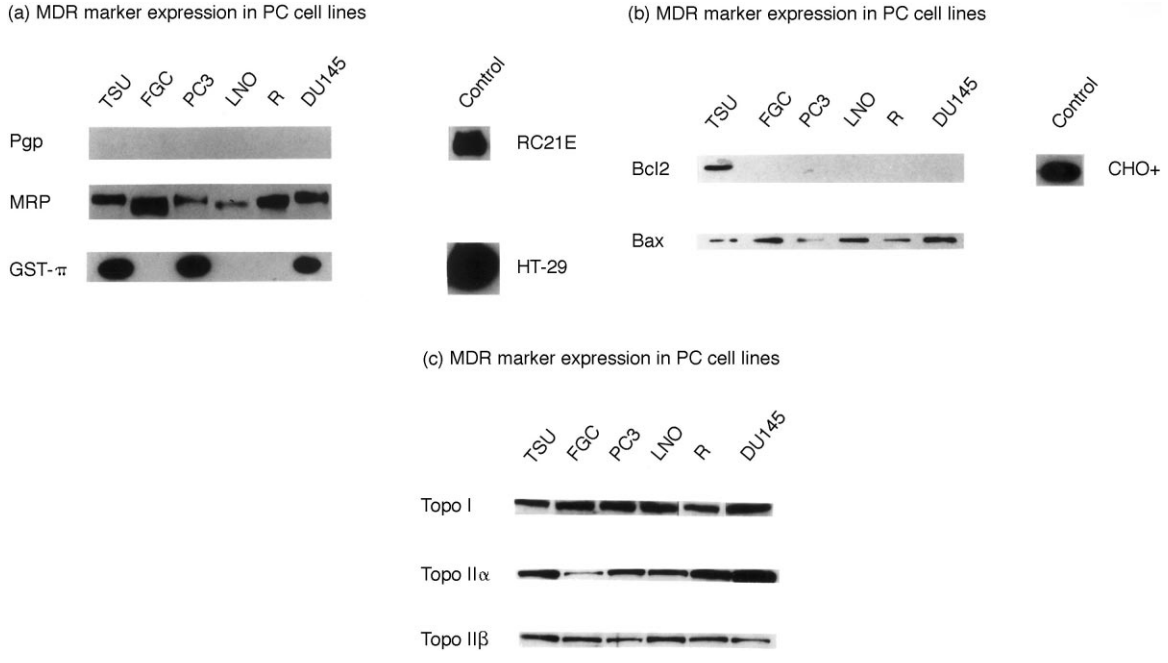


**Figure 2. Expression of multidrug resistance proteins P-glycoprotein (Pgp), multidrug resistance-associated protein (MRP), glutathione-S-transferase-π (GST-π), Bcl-2, Bax, topoisomerase (Topo) I, IIα and IIβ in the human prostate cancer cell lines TSU-Pr1, LNCaP-FGC, PC3, LNCaP-LNO, LNCaP-R and DU145 as determined by Western blotting of total cell lysates. Bands represent protein expression. Positive controls are shown for Pgp (RC21E), GST-π (HT-29) and Bcl-2 (CHO-Bcl-2) detection and were run on the same gel.**

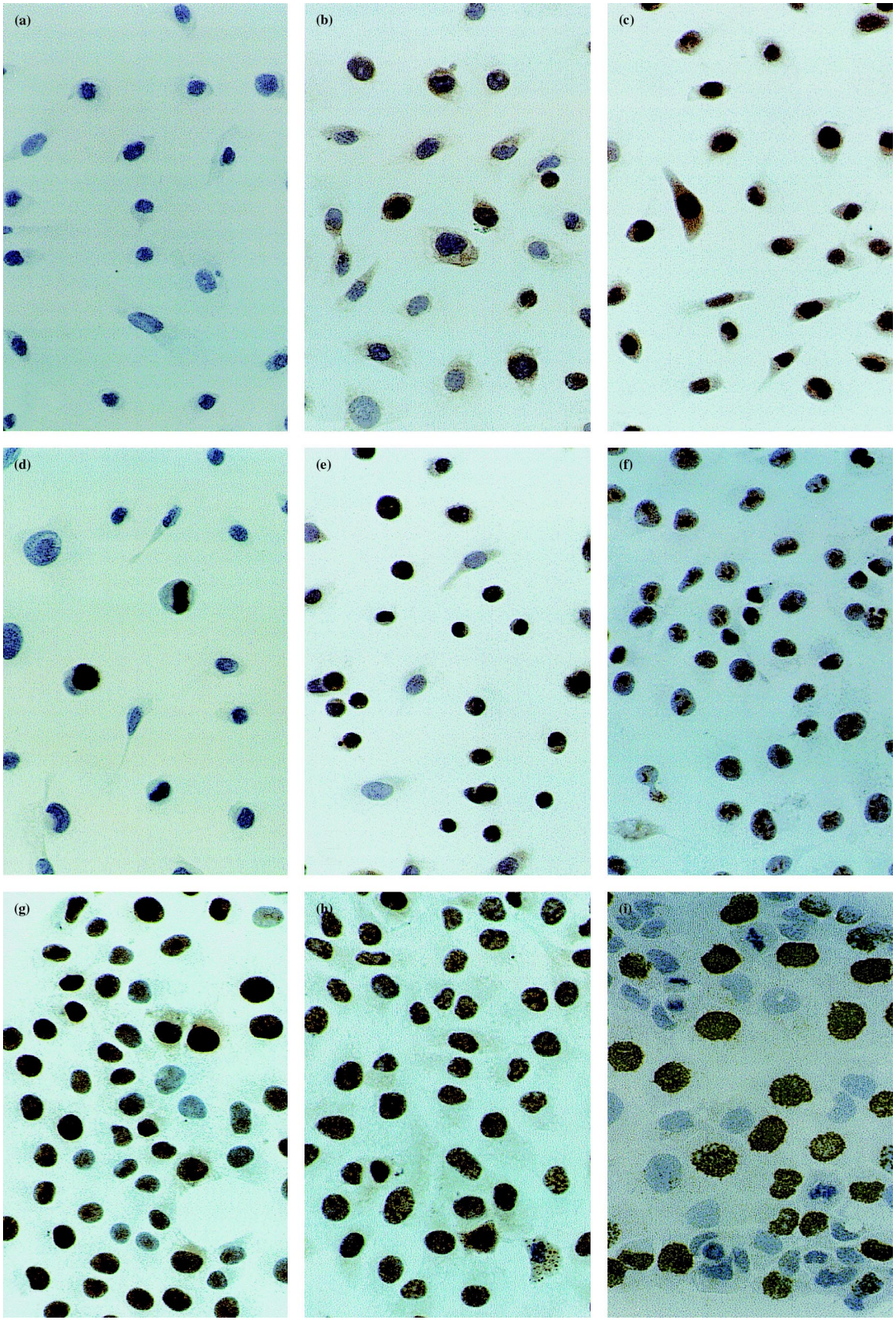


**Figure 3. Immunocytochemical staining of P-glycoprotein (Pgp) in PC3 (a), multidrug resistance-associated protein (MRP) in PC3 (b), glutathione-S-transferase-π (GST-π) in PC3 (c), Bcl-2 in PC3 (d), Bax in PC3 (e), topoisomerase (Topo) I in DU145 (f), Topo IIα in DU145 (g), Topo IIβ in DU145 (h) and bromodeoxyuridine (BrdU) in DU145 (i) (magnification 400×).**

*Table 2. Percentages of cells staining positively for topoisomerase IIα (Topo IIα) and bromodeoxyuridine (BrdU) as determined by immunocytochemistry*

| | TSU-Pr1 | LNCaP-FGC | PC3 | LNCaP-LNO | LNCaP-R | DU145 |
|---|---|---|---|---|---|---|
| Topo IIα | 68 | 40 | 80 | 48 | 33 | 87 |
| BrdU | 41 | 17 | 32 | 18 | 27 | 43 |

cultures of all cell lines were stained with antibodies directed against the MDR proteins (Figure 3). The immunocytochemical stainings matched the Western blotting results. All prostate cancer cell lines were negative for Pgp (Figure 3a), whereas RC21E control cells were clearly Pgp positive, with evident cell membrane-bound localisation (not shown). MRP was expressed in all cell lines, demonstrated as a cytoplasmic staining (Figure 3b). GST-π stained positively in cell lines TSU-Pr1, PC3 (Figure 3c) and DU145 and showed a reticular cytoplasmic pattern and nuclear staining. Bcl-2 was only expressed at a low level in TSU-Pr1. The other cell lines showed some staining of mitotic cells (Figure 3d). All cell lines were Bax positive (Figure 3e). Topo I was present in the nuclei of all cell lines, with prominent nucleolar staining (Figure 3f). Also, a light cytoplasmic background staining was observed. Nuclear and nucleolar Topo IIα was present in all cell lines but varied between cell lines. DU145 (Figure 3g) and TSU-Pr1 cells stained intensely in comparison with the other cell lines. Topo IIα expression was compared with BrdU incorporation of cells (Figure 3i) during the S phase of the cell cycle (Table 2). The BrdU staining results corresponded well with the proliferative status of cells as observed during the MTT assay. Topo IIα expression appeared to be related to the proliferative activity of most of the prostate cancer cell lines. Topo IIβ was expressed in all cell lines with predominant granular staining of the nuclei and absence of staining of nucleoli (Figure 3h) and was expressed in the majority of cells.

## DISCUSSION

To gain insight into the mechanisms involved in the resistance of metastatic hormone-independent prostate cancer to chemotherapy we determined the expression of MDR proteins, which may lead to the MDR phenotype, in prostate cancer cell lines. Furthermore, we assessed the resistance profiles to three commonly used antineoplastic agents, etoposide, doxorubicin and vinblastin, whose action is affected by MDR.

We found that the expression of Pgp, the energy-dependent drug transporter molecule, was below detection levels in all cell lines. The expression of Pgp in the human prostate cancer cell lines PC3 and DU145 has been reported in one study [24], albeit at low levels. Doxorubicin resistant cell lines derived from the Dunning R3327 rat prostate carcinoma model [25] expressed Pgp as well [26]. In contrast, Mickisch and colleagues [27] reported that *MDR1* mRNA was undetectable in prostate cancer cell lines LNCaP and DU145. Exposure of non-*MDR1* expressing prostate carcinoma cells to Pgp targeted immunoconjugates harbouring *Pseudomonas* exotoxin did not affect these cells, whereas Pgp expressing renal cell cancer cell lines were killed. Goldstein and associates [28] were also unable to detect Pgp in clinical prostate cancer or prostate cancer cell lines. The generally negative results for Pgp suggest that Pgp is probably not significantly involved in MDR of prostate cancer.

MRP, the MDR-associated protein, which acts as a transporter molecule for glutathione-S-conjugates [22], was variably expressed at low levels compared with the MRP overexpressing cell line GLC4/ADR. Little is known about the expression and function of the drug transporter MRP in prostate cancer. Low expression levels have been reported in clinical prostate cancer samples by Nooter and colleagues [29]. The expression of MRP in our models indicates that this MDR protein may play a functional role in prostate cancer.

GST-π was expressed in TSU-Pr1, PC3 and DU145. Among the glutathione-S conjugates, which participate in the detoxification of xenobiotics by conjugation to reduced glutathione, the GST-π isoenzyme is especially associated with the resistance of tumours to alkylating agents and cisplatin [30]. GST-π was found in normal prostate tissue, but was absent in locally confined prostate cancer [31, 32]. Our results with GST-π were in line with the observations of Lee and associates [31], who reported that cell lines TSU-Pr1, PC3 and DU145 expressed GST-π, but the LNCaP cells did not. This lack of expression in LNCaP is possibly due to hypermethylation of GST-π promotor sequences [31]. The expression of GST-π in several of our cell lines suggests a potential role of this enzyme in drug resistance of advanced prostate cancer.

Bcl-2 [9] has cell survival promoting capacity and blocks cancer cells in their ability to undergo drug-induced apoptosis (e.g. by etoposide). In our cell lines, Bcl-2 was only expressed in TSU-Pr1. Conflicting data have been reported about the expression of Bcl-2 in LNCaP, but generally the expression of this protein was low or undetectable. The expression of Bcl-2 in PC3 has been reported by Sinha and colleagues [33], in contrast to our findings. Differences between cell lines originating from one parental cell line may have arisen after culturing in different laboratories. In clinical prostate cancer, Bcl-2 appears to be expressed at relatively low levels in low grade carcinomas and at higher levels in high grade tumours [34]. The expression of Bcl-2 in one of our prostate cancer cell lines, together with the results of several studies [33, 35] reporting modulation of drug resistance with changed Bcl-2 expression, support the idea that Bcl-2 contributes to the MDR phenotype of prostate cancer. The role of Bcl-2 in MDR of prostate cancer clearly requires further investigation.

Bax [10] is an apoptosis promoting protein and acts as an opponent of Bcl-2. Bax was expressed in all cell lines tested, and was also found to be well expressed in most cancers including prostate cancer [34]. The ratio of Bcl-2 and Bax in prostate cancer cells may be of importance to determine if the apoptotic cascade will be triggered by treatment with chemotherapy.

The Topo IIα enzyme was expressed in all cell lines. The highest expression was found in the rapidly proliferating cell lines TSU-Pr1 and DU145. Topo I and IIβ were equally expressed in all cell lines. This is in line with the findings of Boege and colleagues [36] that Topo I and IIβ are constantly

expressed during the cell cycle, whereas IIα is proliferation associated. The Topo enzymes are essential for several cellular processes and constitute targets for a number of clinically important drugs, which induce lethal DNA damage by irreversibly stabilising Topo–DNA complexes. Topo directed drugs, such as etoposide and doxorubicin, are generally believed to target the IIα isotype [36], although Topo I [37] and IIβ [38] may constitute targets as well. Changes in the amount or activity of Topo have been described in relation to one form of MDR: atypical or attenuated MDR [11]. The relationship between the resistance of prostate cancer to chemotherapy and the expression of Topo enzymes is still unclear. However, drug resistance to Topo poisons may arise from decreased expression or activity of the drug targets Topo I, IIα and/or IIβ or reduced proliferative activity related to Topo IIα.

The presence of several MDR proteins in the prostate cancer cell lines suggests that drug resistance may arise by up- or downregulation of one or multiple MDR proteins. In our experiments, the cell lines responded variably to exposure to cytotoxic drugs (Figure 1a–c, Table 1). It is likely that this response is influenced by a combination of MDR mechanisms, rendering interpretation of the dose–response curves difficult. However, certain patterns can be distinguished. The cell line TSU-Pr1 was sensitive to all three drugs. Its sensitivity to vinblastin, a mitotic spindle blocker, can be expected, as TSU-Pr1, together with DU145, was one of the most rapidly proliferating cell lines among our models, as measured by BrdU incorporation (Table 2). The sensitivity of TSU-Pr1 to etoposide and doxorubicin, both Topo II targeting drugs, relates well to the high expression of Topo IIα and β enzymes. TSU-Pr1 expressed Bcl-2, which may protect these cells from etoposide-mediated apoptosis [35]. However, immunocytochemistry showed a heterogenous expression of Bcl-2 and the majority of cells did not express the protein, suggesting that only a small proportion of the cells may have been relatively resistant. DU145, which had high expression of Topo IIα, was also sensitive to etoposide and doxorubicin. The sensitivity of LNCaP-FGC, having a lower expression of Topo IIα and a lower proliferative activity, was nearly identical to that of DU145. Possibly GST-π, the detoxifying enzyme, induced a certain degree of drug resistance in DU145, PC3 and TSU-Pr1, whereas LNCaP-FGC, which did not express GST-π, remained relatively sensitive, resulting in similar resistance profiles of these cell lines. The LNCaP derivatives LNCaP-LNO and LNCaP-R, which in contrast to LNCaP-FGC, are both hormone-independent, were relatively resistant to etoposide, doxorubicin and vinblastin. This may be in agreement with the finding that transition of prostate cancer cells to a hormone-independent state concomitantly leads to resistance to chemotherapy [35, 39]. The proliferative activity of the cell line LNCaP-R was higher than that of LNCaP-LNO and its sensitivity to Vinblastin was comparable to that of PC3, DU145 and FGC. The role of MDR in this cell line is questionable as it lacks Pgp, significant amounts of MRP, GST-π and Bcl-2 and expresses Bax and all Topo. The slow growth rate of LNCaP-LNO may explain its resistance to vinblastin.

This study showed that the resistance of prostate cancer cells to chemotherapy may be determined by MDR mechanisms and proliferative activity. Furthermore, drug resistance is potentially multifactorial, as several MDR proteins were expressed in our models. As the provided evidence of the contribution of MDR-associated proteins to drug resistance in prostate cancer is indirect, the development of MDR models from relatively sensitive prostate cancer cell lines is an obligatory step for future investigations. Such models offer the possibility of further functional studies of regulation of activity and expression of MDR proteins *in vitro*. Also, modulation and circumvention of MDR could be investigated. Furthermore, a detailed search for expression of MDR proteins in clinical prostate cancer samples at different stages of disease and progression may help elucidate the role of the various MDR-related factors, which were described in the present study of experimental systems. Eventually, a better understanding of the mechanisms causing resistance of prostate cancer to antineoplastic agents may lead to new approaches to treat hormone-independent metastatic prostate cancer more successfully.


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